

## Products of the isoprostane pathway: unique bioactive compounds and markers of lipid peroxidation

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**Abstract.** We previously reported the discovery of prostaglandin  $F_2$ -like compounds ( $F_2$ -isoprostanes) formed by nonenzymatic free-radical-induced peroxidation of arachidonic acid. Quantification of  $F_2$ -isoprostanes has proven to be a major advance in assessing oxidative stress status in vivo. Central in the pathway of formation of isoprostanes are prostaglandin  $H_2$ -like endoperoxides, which also undergo rearrangement in vivo to form E-ring, D-ring, and thromboxane-ring compounds.  $E_2$ - and  $D_2$ -isoprostanes also undergo dehydration in vivo to form reactive cyclopentenone  $A_2$ - and  $J_2$ -isoprostanes, which are susceptible to Michael addition reactions with thiols. Re-

cently, we described the formation of highly reactive  $\gamma$ -ketoaldehydes (now termed isoketals) as products of isoprostane endoperoxide rearrangement which readily adduct to lysine residues on proteins and induce cross-links at rates that far exceed other aldehyde products of lipid peroxidation. Isoprostane-like compounds (neuroprostanes) and isoketal-like compounds (neuroketals) are formed from oxidation of docosahexaenoic acid, which is enriched in the brain, and measurement of neuroprostanes may provide a unique marker of oxidative neuronal injury.

**Key words.** Isoprostanes; lipid peroxidation; free radicals; protein adducts; isolevuglandins; mass spectrometry.

### Biochemistry of the formation of isoprostanes

One of the major targets of free radicals are polyunsaturated lipids, which undergo peroxidation reactions. A plethora of products are produced by free-radical-induced lipid peroxidation [1, 2]. In 1990, we reported the discovery that a series of novel prostaglandin (PG)  $F_2$ -like compounds are produced by free-radical-induced peroxidation of arachidonic acid in vivo [3]. The mechanism by which these compounds are formed is shown in figure 1. Because these compounds are isomeric to  $PGF_{2\alpha}$  derived from the cyclooxygenase, they have been termed  $F_2$ -isoprostanes ( $F_2$ -IsoPs). A unique aspect of the generation of IsoPs is that they are initially formed in situ on phospholipids and then released preformed by phospholipases [4].

As noted in figure 1, three arachidonyl radicals give rise to four  $F_2$ -IsoP regioisomers. Each of these regio-

isomers comprise eight racemic diastereomers. Each regioisomer is designated by the carbon number on which the side-chain hydroxyl group is located; the carboxyl carbon is designated C-1. This is accordance with the official nomenclature system established for IsoPs that has been approved by the Eicosanoid Nomenclature Committee, sanctioned by the Joint Commission of Biochemical Nomenclature (JBCN) of the International Union of Pure and Applied Chemistry (IUPAC) [5]. Studies carried out to establish the relative abundance of the different  $F_2$ -IsoP regioisomers indicate that there is no preferential formation of regioisomers or isomeric compounds within a regioisomer group than what would be predicted from a nonenzymatic mechanism [6].

Central in the pathway of formation of IsoPs are  $PGH_2$ -like endoperoxides. The  $H_2$ -IsoP endoperoxides can be reduced to form F-ring IsoPs (fig. 1). Recently, we reported the discovery that glutathione is an important effector of the reduction of IsoP endoperoxides to  $F_2$ -IsoPs [7].  $PGH_2$  is an unstable molecule in aqueous solution which under-

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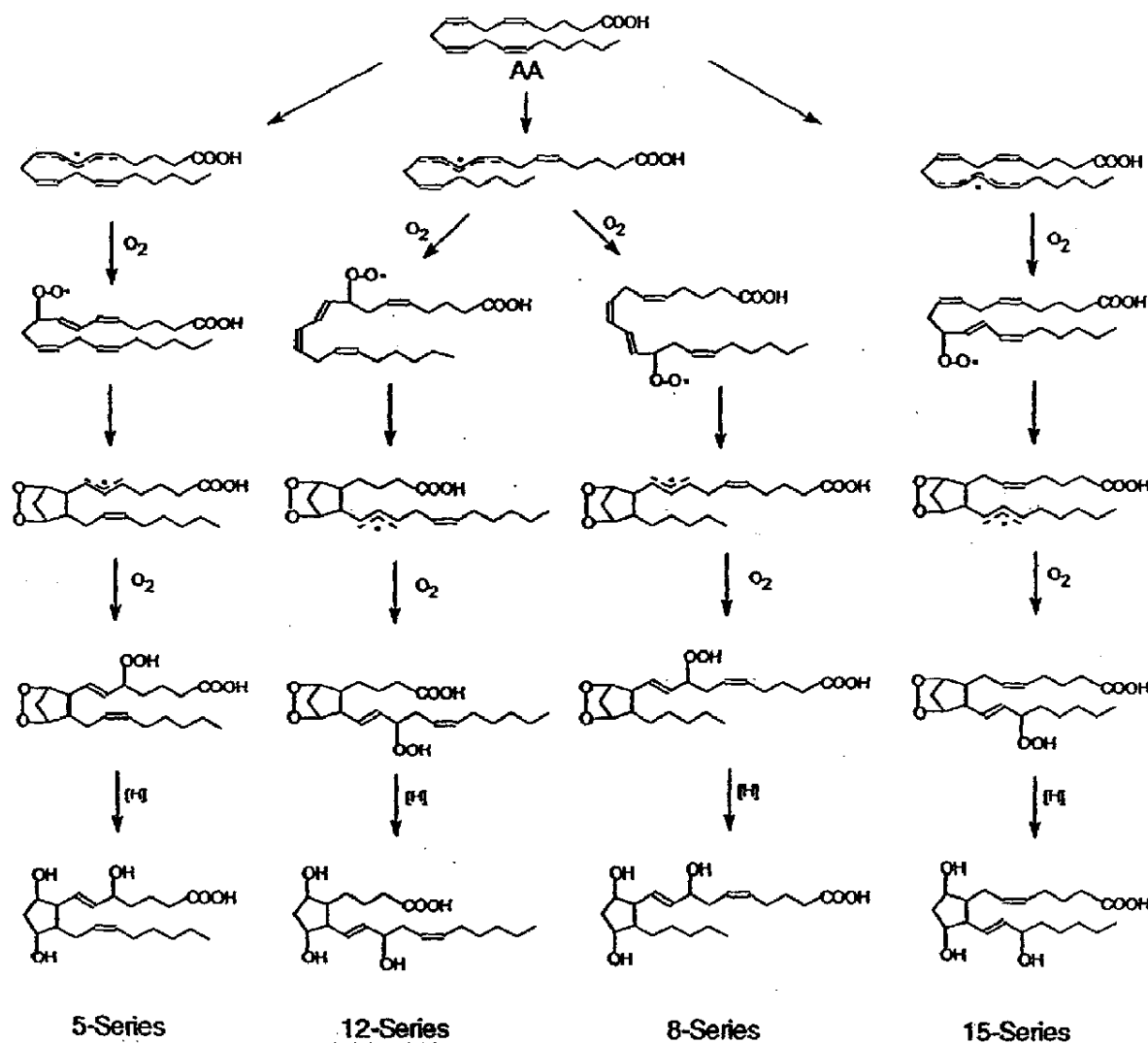


Figure 1. Pathway of formation of F<sub>2</sub>-isoprostanes.

goes rearrangement to form PGE<sub>2</sub> and PGD<sub>2</sub> with a *t*<sub>1/2</sub> of approximately 5 min [8]. In this regard, we explored the possibility that the reduction of the H<sub>2</sub>-IsoP endoperoxides to F<sub>2</sub>-IsoPs may not be completely efficient and found that abundant quantities of E<sub>2</sub>/D<sub>2</sub>-IsoPs are also produced *in vivo* [9]. In addition, PGH<sub>2</sub> may also undergo rearrangement to form a thromboxane ring, and we have found that isothromboxanes are also produced *in vivo* [10].

#### Measurement of F<sub>2</sub>-IsoPs as a marker of lipid peroxidation *in vivo*

Although the initial discovery of F<sub>2</sub>-IsoPs was a curious finding that elucidated new products of lipid peroxida-

tion, the importance of this discovery has evolved considerably over the last few years and encompasses several aspects of the biology and biochemistry of IsoPs. Free radicals have been hypothesized to play an important role in the pathogenesis of a wide variety of disease processes [11, 12]. However, a major impediment in translating these hypotheses to fact has been the lack of a reliable noninvasive approach to assess oxidative stress status *in vivo* in humans [13]. In this regard, quantification of F<sub>2</sub>-IsoPs has proven to be a major advance in this area [14–17].

The method we routinely use for measurement of F<sub>2</sub>-IsoPs is gas chromatography (GC) negative-ion chemical ionization (NICI) mass spectrometry (MS). This method is very sensitive (lower limit of detection ~ 10 femtomol) and has a high degree of precision (± 6%) and accuracy

(96%) [18]. However, it requires expensive instrumentation, and sample processing is relatively labor intensive. Immunoassay kits for one of the  $F_2$ -IsoPs, 15- $F_2$ -IsoP (8-iso-PGF<sub>2a</sub>), are available commercially from several vendors. However, interfering substances can be problematic, as has been recognized with immunoassays for other prostanoids for many years [19]. More often than not, samples have to be subjected to some degree of purification prior to performing the immunoassay. In some instances, extraction of samples without additional purification by thin-layer chromatography (TLC) or high-pressure liquid chromatography (HPLC) can actually concentrate interfering substances. Recently, a direct comparison of GC/MS and enzyme immunoassay (EIA) for  $F_2$ -IsoPs revealed significant inconsistencies between the two methods [20]. Thus, MS remains the most reliable and accurate method for analysis of IsoPs.

To illustrate the comparative advantage of measuring  $F_2$ -IsoPs over other measures of lipid peroxidation to assess oxidant injury, we have directly compared measurements of  $F_2$ -IsoPs with both malondialdehyde (MDA) and lipid hydroperoxides, two of the most commonly used markers of lipid peroxidation, both in vitro and in vivo. The time-course of formation of  $F_2$ -IsoPs during oxidation of liver microsomes correlated very well with the formation of MDA and with the formation of lipid hydroperoxides during oxidation of low density lipoprotein in vitro [21, 22]. However, the fold increase above baseline in  $F_2$ -IsoP levels that occurred in vivo in liver following administration of CCl<sub>4</sub> to rats to induce an intense oxidant injury greatly exceeded that of MDA and lipid hydroperoxides by as much as 26-fold [22, 23]. This indicates that measurement of  $F_2$ -IsoPs is far superior to measurement of MDA and lipid hydroperoxides as an index of oxidative stress in vivo, notwithstanding the fact that MDA is not a specific marker of lipid peroxidation [13, 24].

Measurement of free  $F_2$ -IsoPs in plasma can provide an index of total body production of IsoPs, whereas measurement of levels of  $F_2$ -IsoPs esterified in tissues can localize oxidant injury directly to specific sites of interest. Although sampling of tissues for analysis is primarily limited to experimental animals or post-mortem human tissue samples, analysis of levels esterified in lipoproteins in plasma has been utilized to obtain valuable information regarding the low density lipoprotein (LDL) oxidation hypothesis of atherogenesis in humans [25–28]. One of the potential problems with analysis of IsoPs in plasma is that blood drawing is somewhat invasive and inconvenient, and artifactual generation of IsoPs can occur unless special precautions are used to prevent it [18]. In addition, because the circulating  $t_{1/2}$  of  $F_2$ -IsoPs in the circulation is only approximately 16 min [20], there is also a potential drawback with measuring plasma concentrations of  $F_2$ -IsoPs in that it only provides an index of IsoP production at a single limited point in time. This may

be problematic in certain disease states in which there may be significant intraday fluctuations in the magnitude of IsoP production. Analysis of urine for unmetabolized  $F_2$ -IsoPs also has some limitations as an index of systemic production of IsoPs, because there appears to be some contribution to free levels of unmetabolized  $F_2$ -IsoPs in urine from local formation of IsoPs in the kidney which are excreted directly into the urine [14].

These potential limitations with measurements of unmetabolized  $F_2$ -IsoPs can be overcome by measurement of the urinary excretion of a metabolite of  $F_2$ -IsoPs. This is because (i) metabolism of prostanoids occurs predominantly in extrarenal tissues, (ii) urine collected over several hours can provide an integrated index of IsoP production over time, (iii) collection of urine for analysis is noninvasive, and (iv) IsoP metabolites cannot be generated artifactually *ex vivo* by autooxidation. Toward this goal, we recently reported the identification of the major urinary metabolite of the  $F_2$ -IsoP, 15- $F_2$ -IsoP, in humans as 2,3-dinor-5,6-dihydro-15- $F_2$ -IsoP (fig. 2) [29] and recently developed a highly accurate stable isotope dilution GC/MS assay for this metabolite [30]. It is anticipated that the measurement of the urinary excretion of this metabolite will prove to be an important advance in assessing oxidative stress status in vivo in humans that will be applicable to large clinical studies.

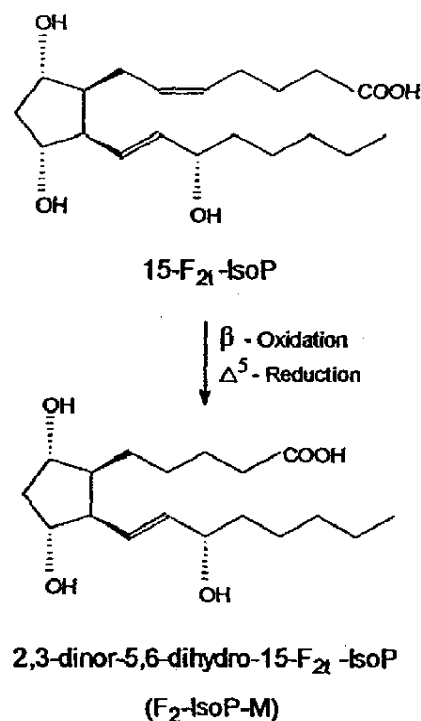


Figure 2. Formation of the major urinary metabolite of 15- $F_2$ -IsoP by one step of  $\beta$ -oxidation and reduction of the  $\Delta^5$  double bond.

Measurements of endogenous  $F_2$ -IsoP production have provided compelling evidence for a role for free radicals, often for the first time, in the pathogenesis of a remarkably large number of diverse disease processes. These range from cigarette smoking [28, 31], renal failure of rhabdomyolysis [32, 33], renal failure associated with aging [34], hepatorenal syndrome [35], scleroderma [36], CCL<sub>4</sub>-induced hepatotoxicity [3, 37], diquat-induced hepatotoxicity [3, 38], oxidative modification of LDL and atherogenesis [22, 25–28, 39–41], vitamin E deficiency [42], selenium deficiency [42], halothane-induced hepatotoxicity [43], organophosphate poisoning [44], Alzheimer's disease [45–47], Huntington's disease [48], hyperhomocysteinemia [49], acute Cr (VI) poisoning [50], cisplatin renal injury [51], diabetes [52–54], ischemia/reperfusion injury [25, 55–58], alcoholic liver disease [59, 60], acute cholestasis [61, 62], adult respiratory distress syndrome [63] in addition to data suggesting that  $F_2$ -IsoP production has predictive value for survival in this syndrome [unpublished], chronic obstructive lung disease [64], interstitial lung diseases [65], cold preservation of transplantable kidneys [66] and allergic asthma [67]. The reader is referred to the original articles for specifics regarding the data obtained.

### **Isoprostanes as bioeffectors of oxidant injury**

In addition to being reliable markers of lipid peroxidation *in vivo*, IsoPs can also exert potent biological activity and potentially mediate some of the adverse effects of oxidant injury. First, as mentioned, IsoPs are initially formed esterified on phospholipids. Molecular modelling of IsoP-containing phospholipids reveals them to be remarkably distorted molecules [4]. Thus, the formation of these abnormal phospholipids would be expected to exert profound effects on membrane fluidity and integrity, well-known sequelae of oxidant injury.

### **Receptor-mediated biological actions**

One of the unique characteristics of IsoPs that contrasts with prostaglandins formed by the cyclooxygenase enzyme is that the side chains are predominantly oriented *cis* in relation to the cyclopentane ring [68]. Two IsoPs that have been available for biological testing are 15- $F_2$ -IsoP (8-iso-PGF<sub>2α</sub>) and 15- $E_2$ -IsoP (8-iso-PGE<sub>2</sub>), which differ from their respective counterparts derived from the cyclooxygenase by inversion of the upper side chain stereochemistry. We have previously shown that both 15- $F_2$ -IsoP and 15- $E_2$ -IsoP are produced *in vivo* [69, 70]. Both of these IsoPs have been shown to be potent vasoconstrictors in a variety of vascular beds, including the kidney [3, 9, 71, 72], lung [73, 74], heart [75], retina [76], portal vein [77], brain [78] and also lymphatics [79]. In

addition, 15- $F_2$ -IsoP induces endothelin release and proliferation of vascular smooth muscle cells [80, 81]. Results from initial experiments suggested that the vascular effects of both of these IsoPs may result from an interaction with thromboxane receptors based on the finding that the vasoconstriction could be abrogated by thromboxane receptor antagonists [71]. However, a number lines of evidence obtained subsequently suggests that these IsoPs may not interact with thromboxane receptors [72, 76, 82–85]. Whether these IsoPs mediate their effects by interaction with some other known receptor(s) or a novel 'IsoP receptor(s)' remains to be determined. Interestingly, in cerebral and retinal vasculature, we recently found that 15- $F_2$ -IsoP induces the formation of thromboxane, which mediates its contractile effects in these vascular beds [86, 87]. Induction of thromboxane formation by 15- $F_2$ -IsoP has not been found in other vascular beds [71]. (12-iso-PGF<sub>2α</sub>) has also become available for biological testing, and has been found to activate the PGF<sub>2α</sub> receptor at relatively high concentrations and to induce hypertrophy of cardiac smooth muscle cells [88, 89]. Metabolism of prostanoids is important mechanism for bioinactivation. However, of considerable interest was our recent novel finding that the major urinary metabolite of 15- $F_2$ -IsoP, 2,3-dinor-5,6-dihydro-15- $F_2$ -IsoP, is also a potent vasoconstrictor [90]. This is also interesting from another perspective in that this metabolite can also be formed by direct oxidation of  $\gamma$ -linolenic acid. The forthcoming availability of additional compounds for biological testing will likely contribute in a valuable way to our understanding of receptor-mediated actions of IsoPs as effectors of oxidant injury.

### **Receptor-independent biological effects of compounds generated by the IsoP pathway**

Recently we reported the discovery of two new groups of novel compounds that are capable of exerting biological effects due to their inherent chemical reactivity. One group of compounds are cyclopentenone IsoPs [91]. These compounds are formed by dehydration of  $E_2$ -isoPs and  $D_2$ -IsoPs (fig. 3), analogous to the formation of PGA<sub>2</sub> and PGJ<sub>2</sub> by dehydration of cyclooxygenase-derived PGE<sub>2</sub> and PGD<sub>2</sub>, respectively. Therefore, these cyclopentenone IsoPs are termed A<sub>2</sub>/J<sub>2</sub>-IsoPs. As with the other IsoP series of compounds, four regioisomers of both A-ring and J-ring IsoPs are formed. The unique feature of these compounds is that they are  $\alpha,\beta$ -unsaturated carbonyls, which confers reactivity, in particular rendering them highly susceptible to Michael addition reactions [92–94]. Cyclopentenone prostaglandins derived from the cyclooxygenase have been a subject of considerable interest because of the unique biological actions they exert, which has been attributed to the reactive  $\alpha,\beta$ -unsaturated carbonyl moiety [94]. Specifically, they have

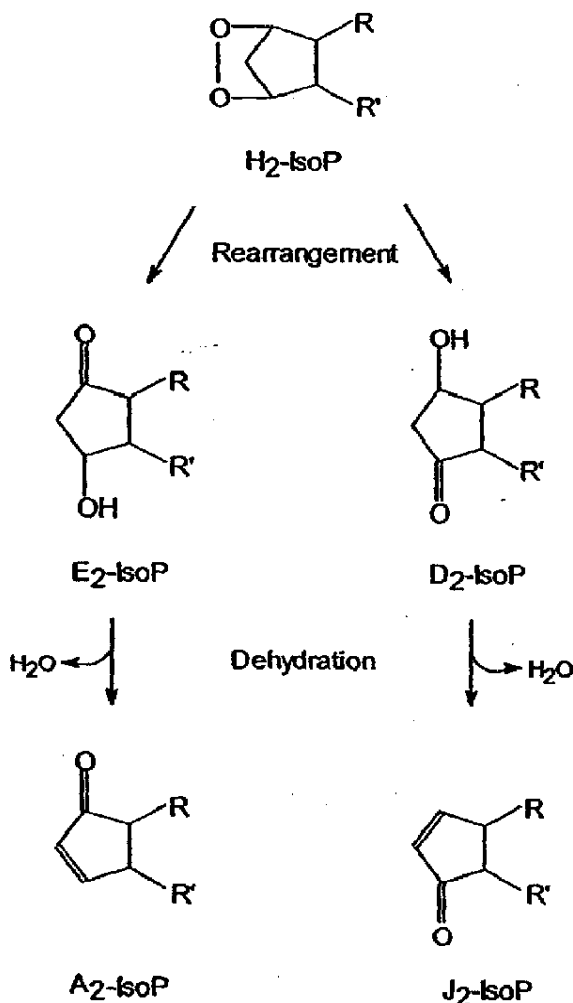


Figure 3. Formation of cyclopentenone A<sub>2</sub>-IsoPs and J<sub>2</sub>-IsoPs by dehydration of E<sub>2</sub>-IsoPs and D<sub>2</sub>-IsoPs, respectively.

been shown to inhibit cellular proliferation and induce differentiation, an effect that may be related to their ability to modulate a variety of growth-related and stress-induced genes [95–97]. These cytostatic effects can be reversible, but higher concentrations are cytotoxic and induce apoptosis [96, 98, 99].

Although the biological effects exerted by PGA<sub>2</sub> and PGJ<sub>2</sub> have been extensively investigated for many years, the extent to which these compounds are formed in vivo has been the subject of continuing controversy for over 2 decades [100–102]. Recently, Δ<sup>12</sup>-PGJ<sub>2</sub> was definitively identified in human urine, but whether this arose from dehydration of PGD<sub>2</sub> in the genitourinary tract prior to voiding or from systemic sources is unclear [91, 103]. Recently, we demonstrated that in normal rat liver, levels A<sub>2</sub>/J<sub>2</sub>-IsoPs could be detected esterified in phospholipids at a level of 5.1 ± 2.3 ng/g liver [91]. In the same livers, levels of E<sub>2</sub>/D<sub>2</sub>-IsoPs were 28.0 ± 4.3 ng/g liver. Following administration of CCl<sub>4</sub> to induce an oxidant injury, levels of A<sub>2</sub>/J<sub>2</sub>-IsoPs and E<sub>2</sub>/J<sub>2</sub>-IsoPs increased strikingly and to a similar extent, 23.9-fold and 21.2-fold, respectively. One of the A<sub>2</sub>-IsoPs, 15-A<sub>2</sub>-IsoP, was found to readily undergo Michael addition with glutathione in the presence of glutathione-S-transferase; approximately 70% had conjugated within 2 min, and the conjugation was complete by 8 min (fig. 4A). In addition, 15-A<sub>2</sub>-IsoP was demonstrated to form covalent adducts with protein, using albumin as a model (fig. 4B). Interestingly, whereas F<sub>2</sub>-, E<sub>2</sub>- and D<sub>2</sub>-IsoPs reach very high levels in the circulation following administration of CCl<sub>4</sub> [3,9,37], A<sub>2</sub>/J<sub>2</sub>-IsoPs could not be detected in free form in the circulation, even following administration of CCl<sub>4</sub>. This can likely be explained by the finding that almost all the radioactivity excreted into urine following administration of radiolabelled 15-A<sub>2</sub>-IsoP to a human volunteer had undergone conjugation to form a polar conjugate, presumably with

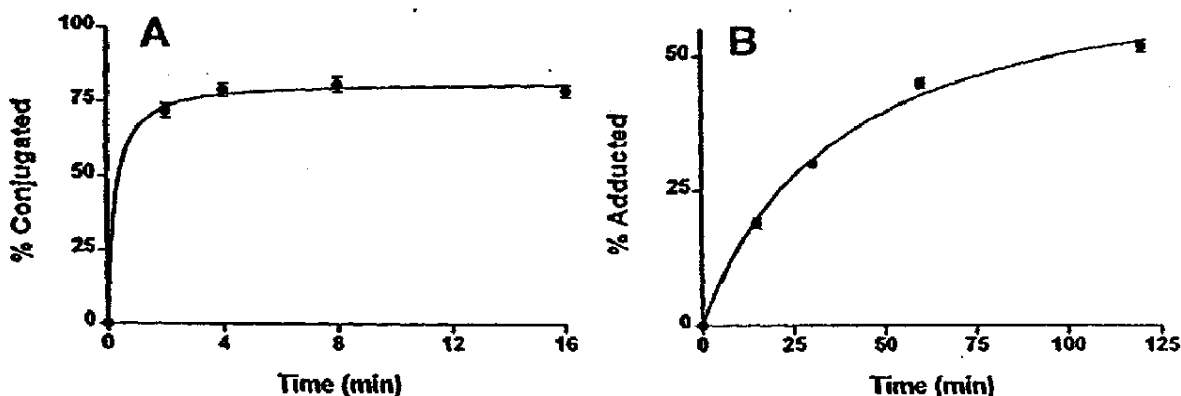


Figure 4. (A) Rate of conjugation of 15-A<sub>2</sub>-IsoP with glutathione in the presence of glutathione-S-transferase. (B) Rate of covalent adduction of 15-A<sub>2</sub>-IsoP with albumin.

glutathione [91]. These data are consistent with our previous findings indicating that formation of polar conjugates is a major pathway of metabolic disposition of  $\Delta^12$ -PGJ<sub>2</sub> in the rat [104]. In summary, these studies have elucidated a new class of reactive compounds formed as products of the IsoP pathway that are capable of exerting biological effects relevant to the pathogenesis of oxidant injury.

We recently reported the discovery of another class of even more highly reactive compounds, acyclic  $\gamma$ -ketoaldehydes, that are formed as products of the IsoP pathway [105]. In 1985, Salomon and colleagues described the formation of  $\gamma$ -ketoaldehydes from rearrangement of PGH<sub>2</sub> derived from the cyclooxygenase in vitro [106]. Because of the structural similarities to levulinialdehyde, these compounds were termed levuglandins (LGs) E<sub>2</sub> and D<sub>2</sub>. Thus, we explored the hypothesis that analogous  $\gamma$ -ketoaldehydes, which we now term E<sub>2</sub>- and D<sub>2</sub>-isoketals (IsoKs) to distinguish them from LGs formed by the cyclooxygenase, may also be formed by rearrangement of H<sub>2</sub>-IsoP endoperoxides (fig. 5). Because, as shown in figure 1, there are four H<sub>2</sub>-IsoP endoperoxide regioisomers, four regioisomers of both E<sub>2</sub>- and D<sub>2</sub>-IsoKs are also formed.

Oxidation of arachidonic acid in vitro yielded a series of compounds that were confirmed to be IsoKs using a number of mass spectrometric approaches, including electron impact mass spectral analysis. Interestingly, the amounts IsoKs formed in vitro were found to be intermediate between the amount of F<sub>2</sub>-IsoPs and E<sub>2</sub>/D<sub>2</sub>-IsoPs formed, indicating that these compounds are formed in relevant quantities compared with other IsoPs. Nonetheless, we could not detect their formation in biological systems in

vitro, i.e. oxidation of liver microsomes and LDLs, nor could we detect them in plasma, urine, or in the circulation or liver following administration of CCl<sub>4</sub> to rats. We hypothesized that this may be due to very rapid adduction to proteins. In this regard, it should be mentioned that other reactive aldehydes that are generated as products of lipid peroxidation, e.g. 4-hydroxynonenal and malondialdehyde, can be detected in their free nonadducted form in biological fluids and tissues [107]. To obtain support for this hypothesis, we assessed the rate of adduction of E<sub>2</sub>-IsoK to protein, using albumin as a model, and compared this with the rate of adduction of 4-hydroxynonenal. The results obtained were most informative. Rate of adduction was determined by assessing the percent decline in free levels of compounds measured in aliquots removed during incubations with albumin over time. As shown in figure 6, E<sub>2</sub>-IsoK underwent adduction with extreme rapidity; 60% had adducted within the first 20 s of incubation. In striking contrast, approximately 50% of 4-hydroxynonenal still remained unadducted after 1 h. Of note is that the free level of E<sub>2</sub>-IsoK does not decline to completely zero but plateaus near zero. This is likely due to the presence of some E<sub>2</sub>-IsoK in which the double bond on the lower side chain has migrated from the  $\Delta^{10}$  to the  $\Delta^9$  position, rendering the molecule less reactive [108]. These data indicate that LGs adduct to protein at a rate that exceeds that of 4-hydroxynonenal, which is considered one of the most reactive aldehydes formed as a product of lipid peroxidation, by several orders of magnitude.

Therefore, we turned to developing methods to detect the formation of IsoKs as protein adducts using liquid chromatography (LC) electrospray ionization (ESI) tandem

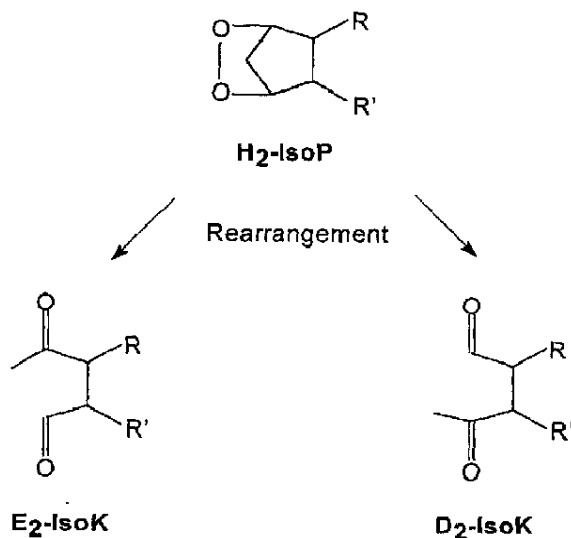


Figure 5. Formation of IsoKs as rearrangement products of IsoP endoperoxides.

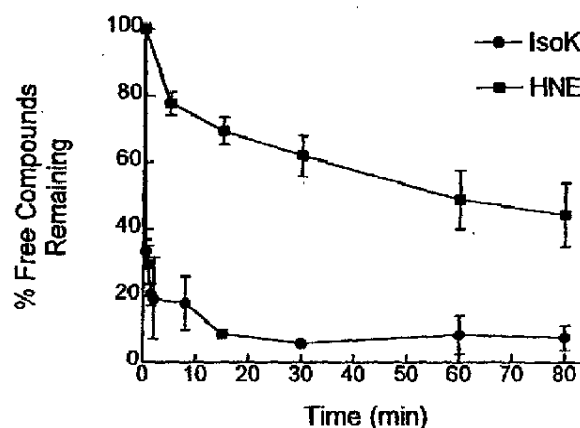


Figure 6. Comparative rates of adduction of E<sub>2</sub>-IsoK and 4-hydroxynonenal during incubation with albumin. Formation of covalent adducts was assessed by the decline in levels of free compounds measured in aliquots removed at various times indicated.

mass spectrometry (MS/MS). Salomon and colleagues had obtained evidence that  $E_2$ -IsoK forms a pyrrole adduct with lysine residues on proteins [109]. However, LC/ESI/MS analysis following incubation of  $E_2$ -IsoK with lysine as a model did not yield evidence for the presence of compounds with the predicted  $MH^+$  ion for a lysyl  $E_2$ -IsoK pyrrole adduct. However, full-spectrum scanning analysis revealed major  $MH^+$  ions present 16 and 32 Da higher than the  $MH^+$  ion for the  $E_2$ -IsoK lysyl pyrrole. These compounds were consistent with lactam and hydroxylactam adducts formed by facile autoxidation of highly substituted pyrroles [110]. This was confirmed by tandem mass spectrometric analyses of adducts formed with various lysine analogs and [ $^{13}C_6$ ] lysine. The analyses for these adducts were performed following prolonged incubation of  $E_2$ -IsoK with lysine. We then analyzed earlier time points, and a new adduct species appeared. This had the appropriate  $MH^+$  ion for a Schiff base adduct. Evidence confirming that this was a lysyl  $E_2$ -IsoK Schiff base adduct was obtained by tandem mass spectrometric analysis and the finding that expected products were formed following treatment with NaCN, methoxyamine  $HCl$ , and  $NaBH_4$  [111].

The time course of formation of these various adducts was then assessed (fig. 7). As noted, the Schiff base adduct is formed very rapidly but is unstable and disappears over time. In contrast, the lactam adducts accumulate much more slowly. The time course of formation of these adducts is consistent with the proposed mechanism of adduct formation depicted in figure 8.  $E_2$ -IsoK and lysine are initially converted via an intermediate to a Schiff base adduct, which is reversible. This intermediate also proceeds through an irreversible pathway leading to the formation of a pyrrole adduct, which then undergoes

autoxidation to form the lactam and hydroxylactam adducts.

With this information in hand, we turned to explore whether we could detect the formation of IsoLG adducts on ApoB protein during oxidation of LDL in vitro. In these experiments, the Apo-B protein was enzymatically digested to individual amino acids and then analyzed for lysyl  $E_2$ -IsoK lactam adducts. This was considered an important experiment in that previously we could not detect the formation of IsoKs in free form during oxidation of LDL. IsoK lactam adducts could not be detected in native LDL, but intense signals were detected for lysyl lactam and hydroxylactam IsoK adducts on ApoB protein following oxidation of LDL [105]. The key question is whether IsoK adducts are formed in vivo. Data obtained from recent experiments indicate that IsoK adducts (i) can be detected in normal rat liver, (ii) levels in rat liver increase significantly following induction of an oxidant injury by administration of  $CCl_4$  and (iii) can be detected in normal human plasma [unpublished].

#### Isoprostane and IsoK-Like compounds from other fatty acids

The basic requirement for cyclization to occur by oxidation of unsaturated fatty acids is the presence of at least three double bonds. Thus, oxidation of linoleic acid (C18:2) does not generate IsoP-like compounds, whereas oxidation of linolenic acid (C18:3) would generate  $F_1$ -IsoPs. However, the relevance of the formation of  $F_1$ -IsoPs is dubious because linolenic acid is only normally present in very minor quantities in vivo.  $F_3$ -IsoPs have recently been described as products of free-radical-induced peroxidation of eicosapentaenoic acid (C20:5) [112]. Again, however, this is not an abundant fatty acid under normal circumstances. The formation of  $F_3$ -IsoPs may be of interest, however, in situations where the ingestion of eicosapentaenoic acid is high, such as high dietary intake of fish and dietary supplementation with fish oil.

Docosahexaenoic acid (DHA) (C22:6) has been a focus of interest because it is present in abundant quantities in the brain, particularly in grey matter, where it comprises up to 25–35% of total fatty acids in aminophospholipids [113, 114]. We recently described the formation of F-ring IsoP-like compounds with four double bonds during free radical-induced peroxidation of DHA in vitro and in vivo [115]. Because DHA is uniquely highly enriched in neurons, we have termed these compounds  $F_4$ -neuroprostanes ( $F_4$ -NPs). Oxidation of DHA leads to the formation of eight  $F_4$ -neuropropane regioisomers, each of which is theoretically comprised of eight racemic diastereomers for a total of 128 compounds (fig. 9). Recently, we have also found that the NP-endoperoxide intermediates also undergo rearrangement to form E-ring

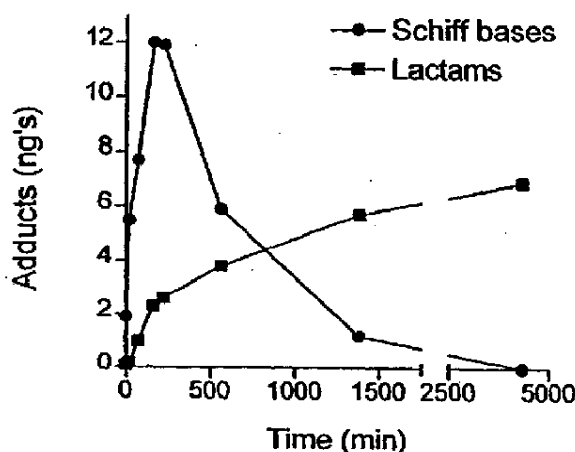


Figure 7. Time course of formation of lactam and Schiff base adducts during incubation of  $E_2$ -IsoK with lysine

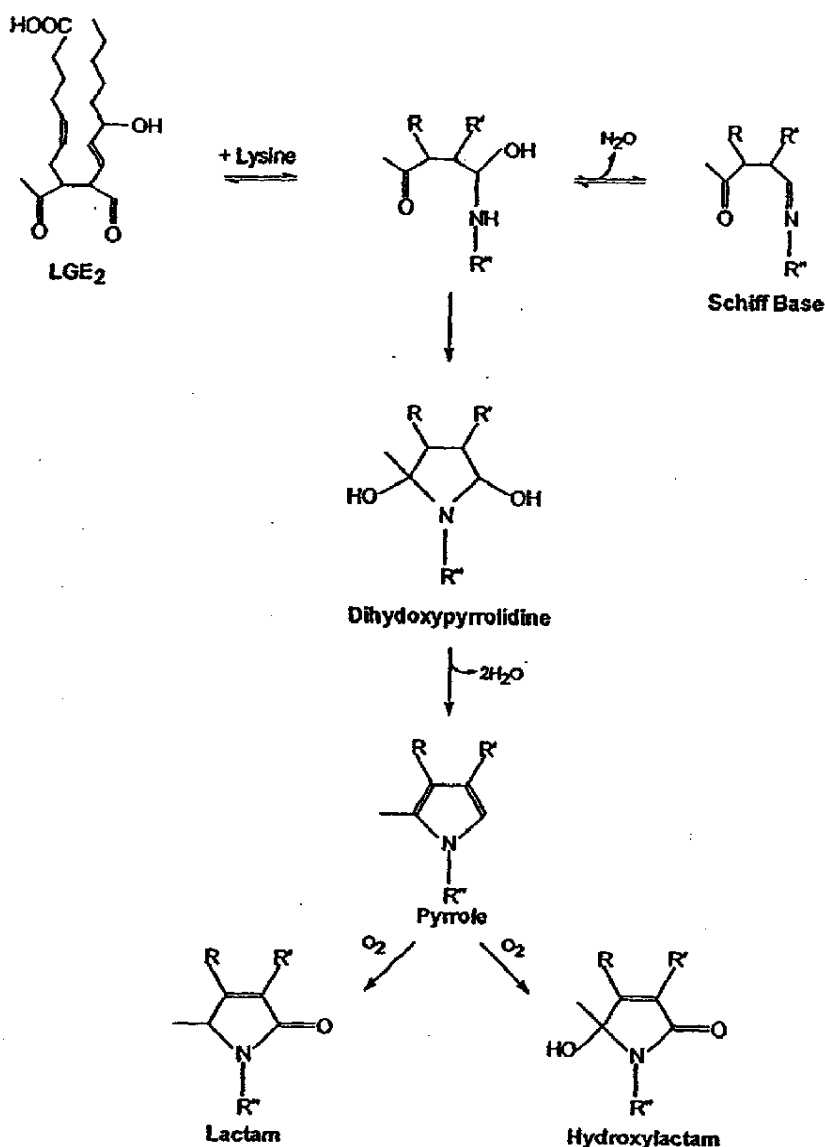


Figure 8. Mechanism of the formation of E<sub>2</sub>-IsoK lysyl Schiff base and lactam adducts.

and D-ring NPs in vivo and that the levels of E<sub>4</sub>/D<sub>4</sub>-NPs esterified in brain of rats slightly exceed the levels of F<sub>4</sub>-NPs [116]. We considered the possibility that measurement of F<sub>4</sub>-neuroprostanes may represent a novel marker of oxidative neuronal injury. Of considerable interest is that we have found that levels of F<sub>4</sub>-NPs are elevated in cerebrospinal fluid from patients with Alzheimer's disease, providing considerable support for a role of free radicals in neuronal injury in this disease [45, 115]. Interestingly, we found an excellent correlation between levels of F<sub>2</sub>-IsoPs and F<sub>4</sub>-NPs in cerebrospinal fluid from patients with Alzheimer's disease and age-matched

normal controls over the range of concentrations present ( $r = 0.88$ ,  $P = 0.0003$ ) [45]. Interestingly, however, only levels of F<sub>4</sub>-NPs, but not F<sub>2</sub>-IsoPs, esterified in brain lipids were found to be increased in Alzheimer's disease [117, 118]. The reason for these differences between the relative increases in tissue versus cerebrospinal concentrations of F<sub>2</sub>-IsoPs and F<sub>4</sub>-NPs remains unclear.

We also recently discovered that IsoK-like compounds (neuroketals, NKs) are also formed in vivo from DHA [119]. Our interest in NKs stems from the possibility that these highly reactive compounds may in part be responsible for cross-linking of proteins in neurodegenerative

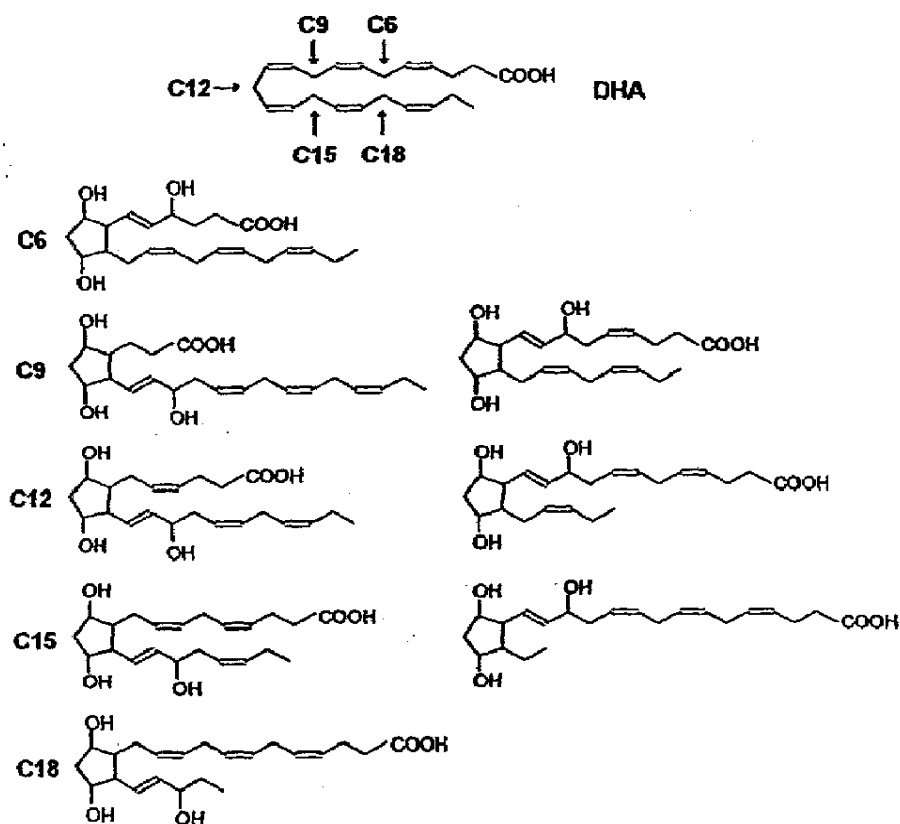


Figure 9. Formation of eight regioisomers of  $F_4$ -neuroprostanes from oxidation of DHA. Abstraction of specific allylic hydrogens that result in the formation of the individual regioisomers are designated by carbon atom numbers (C).

diseases, such as amyloid-beta and tau proteins in Alzheimer's disease.

In summary, the initial discovery of  $F_2$ -IsoPs was of biochemical interest, but this has evolved into a discovery of import for a number of reasons. First, quantification of  $F_2$ -IsoPs has proven to be a major advance in our ability to reliably assess oxidative stress status *in vivo*. Many studies utilizing measurements of  $F_2$ -IsoPs have been able for the first time to strongly implicate an important role for free radicals in many disease processes. Further, our understanding of the biochemistry of the IsoP pathway has expanded greatly. In addition to F-ring IsoPs, we have found that E-ring, D-ring, A-ring, J-ring, thromboxane-ring compounds and acyclic levuglandin-like compounds are also produced *in vivo* as products of the IsoP pathway. This is of considerable interest in that almost the entire spectrum of compounds produced by the cyclooxygenase pathway has now been shown to be produced by a nonenzymatic process. In addition, IsoP-like compounds can also be generated from other unsaturated fatty acids and of particular interest in this regard are NPs formed from oxidation of DHA. IsoPs and related compounds produced by the IsoP pathway are also relevant

not simply from a biochemical perspective but because they can exert potent biological activity. This involves apparent receptor-mediated actions as well as, in the case of cyclopentenone IsoPs and IsoKs, and NKs, actions attributed to inherent chemical reactivity, which can lead to covalent modification of critical biomolecules, i.e. proteins and DNA. Thus, the current understanding of the IsoP pathway has diverged into a variety of areas of potential biochemical and biological importance that is likely to continue to expand as new avenues for scientific inquiry regarding these unique molecules emerge.

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- 1 Gardner H. W. (1989) Oxygen radical chemistry of polyunsaturated fatty acids. *Free Rad. Biol. Med.* 7: 65–86
- 2 Porter N. A., Caldwell S. E. and Mills K. A. (1995) Mechanisms of free radical oxidation of unsaturated lipids. *Lipids* 30: 277–290

- 3 Morrow J. D., Hill K. E., Burk R. F., Nammour T. M., Badr K. F. and Roberts L. J. II (1990) A series of prostaglandin  $F_2$ -like compounds are produced in vivo in humans by a non-cyclooxygenase free radical catalyzed mechanism. *Proc. Natl. Acad. Sci. USA* **87**: 9383–9387
- 4 Morrow J. D., Awad J. A., Boss H. J., Blair I. A. and Roberts L. J. II (1992) Non-cyclooxygenase derived prostanoids ( $F_2$ -isoprostanes) are formed in situ on phospholipids. *Proc. Natl. Acad. Sci. USA* **89**: 10721–10725
- 5 Taber D. F., Morrow J. D. and Roberts L. J. II. (1997) A nomenclature system for the isoprostanes. *Prostaglandins* **53**: 63–67
- 6 Waugh R. J., Morrow J. D., Roberts L. J. II and Murphy R. C. (1997) Identification and relative quantitation of  $F_2$ -isoprostane regioisomers formed in vivo in the rat. *Free Rad. Biol. Med.* **23**: 943–954
- 7 Morrow J. D., Roberts L. J. II, Daniel V. C., Mirotnichenko O., Swift L. and Burk R. F. (1998) Comparison of the formation of  $D_2/E_2$ -isoprostanes to  $F_2$ -isoprostanes in vitro and in vivo: effect of oxygen tension and glutathione. *Arch. Biochem. Biophys.* **353**: 160–171
- 8 Nugteren D. H. and Hazelhof E. (1973) Isolation of properties of intermediates in prostaglandin biosynthesis. *Biochim. Biophys. Acta* **326**: 448–461
- 9 Morrow J. D., Minton T. A., Mukundan C. R., Campbell M. D., Zackert W. E., Daniel V. C. et al. (1994) Free radical induced generation of isoprostanes in vivo: Evidence for the formation of D-ring and E-ring isoprostanes. *J. Biol. Chem.* **269**: 4317–4326
- 10 Morrow J. D., Awad J. A., Wu A., Zackert W. E., Daniel V. C. and Roberts L. J. II (1996) Free radical generation of thromboxane-like compounds (isothromboxanes) in vivo. *J. Biol. Chem.* **271**: 23185–23190
- 11 Halliwell B. and Gutteridge, J. M. C. (1990) Role of free radicals and catalytic metal ions in human disease: an overview. *Methods Enzymol.* **186**: 1–85
- 12 Southorn P. A. and Powis G. (1988) Free radicals in medicine II. Involvement in human disease. *Mayo Clinic Proc.* **63**: 390–408
- 13 Halliwell B. and Grootveld M. (1987) The measurement of free radical reactions in humans. *FEBS Lett.* **213**: 9–14
- 14 Roberts L. J. II and Morrow J. D. (1997) The generation and actions of isoprostanes. *Biochim. Biophys. Acta* **1345**: 121–135
- 15 Morrow J. D. and Roberts L. J. (1996) The isoprostanes: current knowledge and directions for future research. *Biochem. Pharmacol.* **51**: 1–9
- 16 Morrow J. D. and Roberts L. J. II (1997) The isoprostanes: unique bioactive products of lipid peroxidation. *Prog. Lipid Res.* **36**: 1–21
- 17 Moore K. and Roberts L. J. II (1998) Measurement of lipid peroxidation. *Free Rad. Res.* **28**: 659–671
- 18 Morrow J. D. and Roberts L. J. II (1998) Mass spectrometric quantification of  $F_2$ -isoprostanes in biological fluids and tissues as a measure of oxidant stress. *Methods Enzymol.* **300**: 3–12
- 19 Granstrom E. and Kindahl H. (1978) Radioimmunoassay of prostaglandins and thromboxanes. *Adv. Prostaglandin Thromboxane Res.* **5**: 119–210
- 20 Proudfoot J., Barden A., Mori T. A., Burke V., Croft K. D., Beilin L. J. et al. (1999) Measurement of urinary  $F(2)$ -isoprostanes as markers of in vivo lipid peroxidation: a comparison of enzyme immunoassay with gas chromatography/mass spectrometry. *Anal. Biochem.* **272**: 209–215
- 21 Longmire A. W., Swift L. L., Roberts L. J. II, Awad J. A., Burk R. F. and Morrow J. D. (1994) Effect of oxygen tension on the generation of  $F_2$ -isoprostanes and malondialdehyde in peroxidizing rat liver microsomes. *Biochem. Pharmacol.* **47**: 1173–1177
- 22 Lynch S. M., Morrow J. D., Roberts L. J. and Frei B. (1994) Formation of non-cyclooxygenase derived prostanoids ( $F_2$ -isoprostanes) in human plasma and isolated low density lipoproteins exposed to metal ion-dependent and -independent oxidative stress. *J. Clin. Invest.* **93**: 998–1004
- 23 Matthews W. R., McKenna R., Guido D. M., Petre T. W., Jolly R. A., Morrow J. D. et al. (1993) A comparison of gas chromatography-mass spectrometry assays for in vivo lipid peroxidation. *Proc. 41<sup>st</sup> ASMS Conf. Mass Spectrom. Allied Topics*: 865A–865B
- 24 Janero D. R. (1990) Malondialdehyde and thiobarbituric acid-reactivity as diagnostic indices of lipid peroxidation and peroxidative tissue injury. *Free Rad. Biol. Med.* **9**: 515–540
- 25 Roberts L. J. II and Morrow J. D. (1999) Isoprostanes as markers of lipid peroxidation in atherosclerosis. In: *Molecular Biology of Inflammation*, pp 141–163, Serhan C. N. and Ward P. A. (eds), Humana Press, Totowa, NJ
- 26 Gniwotta C., Morrow J. D., Roberts L. J. II and Kuhn H. (1997) Prostaglandin  $F_2$ -like compounds,  $F_2$ -isoprostanes, are present in increased amounts in human atherosclerotic plaques. *J. Arteriosclerosis Thromb. Vasc. Biol.* **17**: 3236–3241
- 27 Pratico D., Iuliano L., Mauriello A., Spagnoli L., Lawson J. A., Rokach J. et al. (1997) Localization of distinct  $F_2$ -isoprostanes in human atherosclerotic lesions. *J. Clin. Invest.* **100**: 2028–2034
- 28 Morrow J. D., Frei B., Longmire A. W., Gaziano J. M., Lynch S. M., Stauss W. E. et al. (1995) Increase in circulating products of lipid peroxidation ( $F_2$ -isoprostanes) in smokers: smoking as a cause of oxidative damage. *N. Engl. J. Med.* **332**: 1198–1203
- 29 Morrow J. D., Awad J. A., Kato T., Takahashi K., Badr K. F., Roberts L. J. II et al. (1992) Formation of non-cyclooxygenase derived prostanoids ( $F_2$ -isoprostanes) in carbon tetrachloride hepatotoxicity: an animal model of lipid peroxidation. *J. Clin. Invest.* **90**: 2502–2507
- 30 Roberts L. J., Moore K. P., Zackert W. E., Oates J. A. and Morrow J. D. (1996) Identification of the major urinary metabolite of the  $F_2$ -isoprostane, 8-iso-prostaglandin  $F_{2a}$ , in humans. *J. Biol. Chem.* **271**: 20617–20620
- 31 Morrow J. D., Zackert W. E., Yang J. P., Kurhts E. H., Callawaert D., Kanai K. et al. (1999) Quantification of the major urinary metabolite of the isoprostane 15- $F_2$ -isoprostane (8-iso-PGF $_{2a}$ ) by stable isotope dilution mass spectrometric assay. *Anal. Biochem.* **269**: 326–331
- 32 Reilly M., Delanty N., Lawson J. A. and FitzGerald G. A. (1996) Modulation of oxidant stress in vivo in chronic cigarette smokers. *Circulation* **94**: 19–25
- 33 Moore K., Patel R., Darley-Usmar V., Holt S., Zackert W. E., Clozel M. et al. (1998) A causative role for redox cycling of myoglobin and its inhibition by alkalization in the pathogenesis and treatment of rhabdomyolysis-induced renal failure. *J. Biol. Chem.* **273**: 31731–31737
- 34 Holt S., Reeder B., Wilson M., Harvey S., Morrow J. D., Roberts L. J. II et al. (1999) Increase lipid peroxidation in patients with rhabdomyolysis. *Lancet* **353**: 1241
- 35 Reckelhoff J. F., Kanji V., Racusen L., Schmidt A. M., Yan S. D., Morrow J. D. et al. (1998) Vitamin E ameliorates enhanced renal lipid peroxidation and accumulation of  $F_2$ -isoprostanes in aging kidneys. *Am. J. Physiol.* **274**: R767–R774
- 36 Morrow J. D., Moore K. P., Awad J. A., Ravenscraft M. D., Marini G., Badr K. F. et al. (1993) Marked overproduction of non-cyclooxygenase derived prostanoids ( $F_2$ -isoprostanes) in the hepatorenal syndrome. *J. Lip. Mediators* **6**: 417–420
- 37 Stein C. M., Awad J. A., Tanner S. B., Roberts L. J. II and Morrow J. D. (1996) Evidence for free radical mediated injury (isoprostane overproduction) in scleroderma. *Arthritis Rheumat.* **39**: 1146–1150
- 38 Awad J. A., Burk R. F. and Roberts L. J. II (1994) Effect of selenium deficiency and glutathione modulating agents on

- diquat toxicity and lipid peroxidation. *J. Pharmac. Exp. Therap.* **270**: 858-864
- 39 Pratico D., Tangirala R. K., Rader D. J., Rokach J. and FitzGerald G. A. (1998) Vitamin E suppresses isoprostane generation in vivo and reduces atherosclerosis in ApoE-deficient mice. *Nat. Med.* **4**: 1189-1192
  - 40 Reilly M. P., Pratico D., Delanty N., DiMinno G., Tremoli E., Rader D. et al. (1998) Increased formation of distinct  $F_2$ -isoprostanes in hypercholesterolemia. *Circulation* **98**: 2822-2828, 1998
  - 41 Moore K. P., Darley-Usmar V., Morrow J. D. and Roberts L. J. II (1995) Formation of  $F_2$ -isoprostanes during the oxidation of human low density lipoprotein and plasma by peroxynitrite. *Circ. Res.* **77**: 335-341
  - 42 Awad J. A., Morrow J. D., Hill K. E., Roberts L. J. II and Burk R. F. (1994) Detection and localization of lipid peroxidation in vitamin E and selenium deficient rats using  $F_2$ -isoprostanes. *J. Nutr.* **124**: 810-864
  - 43 Awad J. A., Horn J. L., Roberts L. J. II and Franks J. J. (1996) Demonstration of halothane-induced hepatic lipid peroxidation in rats using  $F_2$ -isoprostanes. *Anesthesiology* **84**: 910-916
  - 44 Yang Z. P., Wu A., Morrow J. D., Roberts L. J. II and Dettbarn W.-D. (1996) Increases in malondialdehyde-thiobarbituric acid complex (MDA-TBA) and  $F_2$ -isoprostanes in diisopropylfluorophosphate induced muscle hyperactivity. *Biochem. Pharmacol.* **52**: 357-361
  - 45 Montine T. J., Markesbery W. R., Morrow J. D. and Roberts L. J. II. (1998) Cerebrospinal fluid  $F_2$ -isoprostane levels are increased in patients with Alzheimer's disease. *Annals Neurol.* **44**: 410-413
  - 46 Montine T. J., Beal M. F., Cudkovic M. D., O'Donnel H., Margolin R. A., McFarland L. et al. (1999) Increased CSF  $F_2$ -isoprostane concentration in probable AD. *Neurology* **52**: 562-565
  - 47 Pratico D., Lee M. Y., Trojanowski J. Q., Rokach J. and FitzGerald G. A. (1998) Increased  $F_2$ -isoprostanes in Alzheimer's disease: evidence for enhanced lipid peroxidation in vivo. *FASEB J.* **12**: 1777-1783
  - 48 Montine T. J., Beal M. F., Roberts D., Cudkovic M. E., Brown R. H., O'Donnel H. et al. (1999) Cerebrospinal levels of  $F_2$ -isoprostanes, specific markers of lipid peroxidation, are elevated in Huntington's disease patients. *Neurology* **52**: 1104-1105
  - 49 Voutilainen S., Morrow J. D., Roberts L. J. II, Alfhan G., Alho H., Nyssonen K. et al. (1999) Enhanced in vivo lipid peroxidation at elevated plasma homocysteine levels. *Arteriosclerosis Thromb. Vasc. Biol.* **19**: 1263-1266
  - 50 Kadiiska M. B., Morrow J. D., Awad J. A., Roberts L. J. II and Mason R. P. (1998) Enhanced formation of free radicals and  $F_2$ -isoprostanes in vivo by acute Cr (IV) poisoning. *Chem. Res. Toxicol.* **11**: 1516-1520
  - 51 Salahudeen A., Wilson P., Pande R., Poovala V., Kanji V., Ansari N. et al. (1998) Cisplatin induces N-acetyl cysteine suppressible  $F_2$ -isoprostane production and injury in renal tubular epithelial cells. *J. Am. Soc. Nephrol.* **9**: 1448-1455
  - 52 Gopaul N. K., Anggard E. E., Mallet A. I., Betteridge D. J., Wolff S. P. and Norurooz-Zadeh J. (1995) Plasma 8-epi-PGF $_{2\alpha}$  levels are elevated in individuals with non-insulin dependent diabetes mellitus. *FEBS Lett.* **368**: 225-229
  - 53 Natarajan R., Lanting L., Gonzales N. and Nadler J. (1996) Formation of  $F_2$ -isoprostane in vascular smooth muscle cells by elevated glucose and growth factors. *Am. J. Physiol.* **271**: H159-H165
  - 54 Davi G., Ciabattoni G., Consoli A., Mezzetti A., Falco A., Santarone S. et al. (1999) In vivo formation of 8-iso-prostaglandin  $F_{2\alpha}$  and platelet activation in diabetes mellitus: effects of improved metabolic control and vitamin E supplementation. *Circulation* **99**: 224-229
  - 55 Mobert J. and Becker B. G. (1998) Cyclooxygenase inhibition aggravates ischemia-reperfusion injury in the perfused guinea pig heart: involvement of isoprostanes. *J. Am. Coll. Cardiol.* **31**: 1687-1694
  - 56 Reilly M. P., Delanty N., Roy L., Rokach J., Callaghan P. O., Crean P. et al. (1997) Increased formation of isoprostanes IPF $2\alpha$ -I and 8-epi-prostaglandin  $F_{2\alpha}$  in acute coronary angioplasty: evidence for oxidant stress during coronary reperfusion in humans. *Circulation* **96**: 3314-3320
  - 57 Mathews W. R., Guido D. M., Fisher M. A. and Jaeschke H. (1994) Lipid peroxidation as a molecular mechanism of liver cell injury during reperfusion after ischemia. *Free Rad. Biol. Med.* **16**: 763-770
  - 58 Takahashi K., Nammour T. K., Fukunaga M., Ebert J., Morrow J. D., Roberts L. J. II et al. (1992) Glomerular actions of a free radical generated novel prostaglandin, 8-epi-prostaglandin  $F_{2\alpha}$ , in the rat. *J. Clin. Invest.* **90**: 136-141
  - 59 Nanji A. A., Khwaja S., Tahan S. R. and Sadrzadeh S. M. (1994) Plasma levels of a novel non-cyclooxygenase derived prostanoid (8-isoprostane) correlate with severity of liver injury in experimental alcoholic liver disease. *J. Pharmacol. Exp. Therap.* **269**: 1280-1285
  - 60 Alehnik S. I., Leo M. A., Aleynik M. K. and Lieber C. S. (1998) Increased circulating products of lipid peroxidation in patient with alcoholic liver disease. *Alcoholism Clin. Exp. Res.* **22**: 192-196
  - 61 Leo M. A., Aleynik S. I., Siegel J. H., Kasmin F. E., Aleynik M. K. and Lieber C. S. (1997)  $F_2$ -isoprostane and 4-hydroxynonenal excretion in human bile of patients with biliary tract and pancreatic disorders. *Am. J. Gastroenterol.* **92**: 2069-2072
  - 62 Holt S., Marley R., Fernando B., Harry D., Anand R., Goodier D. et al. (1999) Acute cholestasis-induced renal failure: effects of antioxidants and ligands for the thromboxane  $A_2$  receptor. *Kid. Internat.* **55**: 271-277
  - 63 Carpenter C. T., Price P. V. and Christman B. W. (1998) Exhaled breath condensate isoprostanes are elevated in patients with acute lung injury and ARDS. *Chest* **114**: 1653-1659
  - 64 Pratico D., Basili S., Vieri M., Cordova C., Violi V. and FitzGerald G. A. (1998) Chronic obstructive pulmonary disease is associated with an increase in urinary levels of isoprostane F $2\alpha$ -II, an index of oxidant stress. *Am. J. Resp. Crit. Care Med.* **158**: 1709-1714
  - 65 Montuschi P., Ciabattoni G., Paredi P., Pantelidis P., DuBois R. M., Kharitonov S. A. et al. (1998) 8-isoprostane as a biomarker of oxidative stress in interstitial lung diseases. *Am. J. Resp. Crit. Care Med.* **158**: 1524-1527
  - 66 Salahudeen A., Nawaz M., Poovala V., Kanji V., Wang C., Morrow J. D. et al. (1999) Cold induces time-dependent  $F_2$ -isoprostane formation in renal tubular cells and rat kidneys stored in University of Wisconsin solution: implications for immediate post-transplant renal vasoconstriction. *Kidney Internat.* **55**: 1759-1762
  - 67 Dworski R., Murray J. J., Roberts L. J. II, Oates J. A., Morrow J. D., Fisher L. et al. (1999) Allergen-induced synthesis of  $F_2$ -isoprostanes in atopic asthmatics: evidence for oxidative stress. *Am. J. Resp. Crit. Care Med.* **160**: 1947-1951
  - 68 O'Connor D. E., Mihelich E. D. and Coleman M. C. (1984) Stereochemical course of the autooxidative cyclization of lipid hydroperoxides to prostaglandin-like bicyclic endoperoxides. *J. Am. Chem. Soc.* **106**: 3577-3584
  - 69 Morrow J. D., Badr K. F. and Roberts L. J. II (1994) Evidence that the  $F_2$ -isoprostane, 8-epi-PGF $_{2\alpha}$ , is formed in vivo. *Biochim. Biophys. Acta* **1210**: 244-248
  - 70 Morrow J. D., Scruggs J., Chen Y., Zacker W. E. and Roberts L. J. II. (1998) Evidence that the  $E_2$ -isoprostanes 15- $E_2$ -isoprostane (8-iso-prostaglandin  $E_2$ ) is formed in vivo. *J. Lip. Res.* **39**: 1589-1593
  - 71 Takahashi K., Nammour T. M., Fukunaga M., Ebert J., Morrow J. D., Roberts L. J. II et al. (1992) Glomerular actions of

- a free radical-generated novel prostaglandin, 8-epi-prostaglandin  $F_{2a}$ , in the rat. *J. Clin. Invest.* **90**: 136–141
- 72 Fukunaga M., Takahashi K. and Badr K. F. (1993) Vascular smooth muscle actions and receptor interactions of 8-isoprostaglandin  $E_2$ , an  $E_2$ -isoprostane. *Biochem. Biophys. Res. Comm.* **195**: 507–515
  - 73 Kang H. K., Morrow J. D., Roberts L. J. II, Newman J. H. and Banerjee M. (1993) Airway and vascular effects of 8-epi-prostaglandin  $F_{2a}$  in isolated perfused rat lung. *J. Appl. Physiol.* **74**: 460–465
  - 74 Banerjee M., Ho Kang K., Morrow J. D., Roberts L. J. II and Newman J. H. (1992) Effects of a novel prostaglandin, 8-epi-PGF $_{2a}$ , in rabbit lung in situ. *Am. J. Physiol.* **263**: H660–H663
  - 75 Mobert J., Becker B. F., Zahler S. and Gerlach E. (1997) Hemodynamic effects of isoprostanes (8-iso-prostaglandin  $F_{2a}$  and  $E_2$ ) in isolated guinea pig hearts. *J. Cardiovasc. Pharmacol.* **29**: 789–794
  - 76 Lahaie L., Hardy P., Hou X., Hassessian H., Asselin P., Lachapelle P. et al. (1998) A novel mechanism for the vasoconstrictor action of 8-iso-prostaglandin  $F_{2a}$  on retinal blood vessels. *Am. J. Physiol.* **274**: R1406–R1416
  - 77 Marley R., Harry D., Fernando B., Davies S. and Moore K. (1997) 8-iso-prostaglandin  $F_{2a}$ , a product of lipid peroxidation, increases portal pressure in normal and cirrhotic rats. *Gastroenterology* **112**: 208–213
  - 78 Hoffman S. W., Moore S. and Ellis E. F. (1997) Isoprostanes: free radical-generated PGs with constrictor effects on cerebral arteries. *Stroke* **28**: 844–849
  - 79 Sinzinger H., Oguogho A. and Kaliman J. (1997) Isoprostane 8-epi-prostaglandin  $F_{2a}$  is a potent conotractor of human peripheral lymphatics. *Lymphology* **30**: 155–159
  - 80 Fukunaga M., Yura T. and Badr K. F. (1995) Stimulatory effect of 8-epi-PGF $_{2a}$  and  $F_2$ -isoprostane, on endothelin-1 release. *J. Cardiovasc. Pharmacol.* **26** (Suppl. 3) S51–S52
  - 81 Fukunaga M., Makita N., Roberts L. J. II, Morrow J. D., Takahashi K. and Badr K. F. (1993) Evidence for the existence of  $F_2$ -isoprostane receptors on rat vascular smooth muscle cells. *Am. J. Physiol.* **264**: C1619–C1624
  - 82 Morrow J. D., Minton T. A. and Roberts L. J. II. (1992) The  $F_2$ -isoprostane, 8-epi-prostaglandin  $F_{2a}$ , a potent agonist of the vascular thromboxane/endoperoxide receptor, is a platelet thromboxane/endoperoxide receptor antagonist. *Prostaglandins* **44**: 155–163
  - 83 Longmire A. W., Roberts L. J. II and Morrow J. D. (1994) Actions of the  $E_2$ -isoprostane, 8-iso-PGE $_2$ , on platelet thromboxane/endoperoxide receptor in humans and rats: additional evidence for the existence of a unique isoprostane receptor. *Prostaglandins* **48**: 247–256
  - 84 Pratico D., Smyth E. M., Violi F. and FitzGerald G. A. (1996) Local amplification of platelet function by 8-epi-prostaglandin  $F_{2a}$  is not mediated by thromboxane receptor isoforms. *J. Biol. Chem.* **271**: 14916–14924
  - 85 Fukunaga M., Yura T., Grygorczyk R. and Badr K. F. (1997) Evidence for the distinct nature of  $F_2$ -isoprostane receptors from those of thromboxane  $A_2$ . *Am. J. Physiol.* **272**: F477–F483
  - 86 Lahaie O., Hardy P., Hou X., Hassessian H., Asselin P., Lachapelle P. et al. (2001) Cytotoxic effects of thromboxane on neurovascular endothelial cells: Possible role in ischemic neuropathies. *J. Appl. Physiol.* **90**: 279–288
  - 87 Hou X., Gobeil F., Peri K., Speranza G., Marrache M., Lachapelle P. et al. (2000) Differential induction of vasoconstriction and thromboxane formation in immature pig periventricular brain microvessels by 15- $F_2$ -isoprostane (8-iso-prostaglandin  $F_{2a}$ ). *Stroke* **31**: 516–525
  - 88 Kunapuli R., Lawson J. A., Rokach J. and FitzGerald G. A. (1997) Functional characterization of the ocular prostaglandin  $F_{2a}$  receptor. Activation by the isoprostane, 12-iso-PGF $_{2a}$ . *J. Biol. Chem.* **272**: 27147–27154
  - 89 Kunapuli R., Lawson J. A., Meinkoth J. L. and FitzGerald G. A. (1998) Prostaglandin  $F_{2a}$  (PGF $_{2a}$ ) and the isoprostane 8,12-isoprostane  $F_{2a}$ -III, induce cardiomyocyte hypertrophy. Differential activation of downstream signaling pathways. *J. Biol. Chem.* **273**: 22442–22452
  - 90 Hou X., Roberts L. J. II, Morrow J. D., Taber D. F., Kanai K., Gobeil F. et al. (2001) 2,3-Dinor-5,6-dihydro-15- $F_2$ -isoprostane is a bioactive isoprostane metabolite. *Am. J. Physiol.* **281**: R391–R400
  - 91 Chen Y., Morrow J. D. and Roberts L. J. II (1999) Formation of reactive cyclopentenone compounds in vivo as products of the isoprostane pathway. *J. Biol. Chem.* **274**: 10863–10868
  - 92 Boyland E. and Chasseaud L. F. (1968) Enzymes catalyzing conjugations of glutathione with  $\alpha,\beta$ -unsaturated carbonyl compounds. *Biochem. J.* **109**: 651–661
  - 93 Atsmon J., Sweetman B. J., Baertschi S. W., Harris T. M. and Roberts L. J. II (1990) Formation of thiol conjugates of 9-deoxy- $\Delta^9,\Delta^{12}$ (E)-prostaglandin  $D_2$  and  $\Delta^{12}$ (E)-prostaglandin  $D_2$ . *Biochemistry* **29**: 3760–3765
  - 94 Honn K. V. and Marrett L. J. (1985) Requirement of a reactive  $\alpha,\beta$ -unsaturated carbonyl for inhibition of tumor growth and induction of differentiation by 'A' series prostaglandins. *Biochem. Biophys. Res. Commun.* **129**: 34–40
  - 95 Fukushima M. (1992) Biological activities and mechanisms of action of PGJ $_2$  and related compounds: an update. *Prostaglandins Leukotrienes Essent. Fatty Acids* **47**: 1–12
  - 96 Fukushima M. (1990) Prostaglandin  $J_2$ -anti-tumor and antiviral activities and the mechanisms involved. *Eicosanoids* **3**: 189–199
  - 97 Bui T. and Straus D. S. (1998) Effects of cyclopentenone prostaglandins and related compounds on insulin-like growth factor-1 and Waf1 gene expression. *Biochim. Biophys. Acta* **1397**: 31–42
  - 98 Kim I. K., Lee J. H., Sohn H. W., Kim H. S. and Kim S. H. (1993) Prostaglandin  $A_2$  and  $\Delta^{12}$ -prostaglandin  $J_2$  induce apoptosis in L1210 cells. *FEBS Lett.* **321**: 209–214
  - 99 Fukushima M., Kato T., Narumiya S., Mizushima Y., Sasaki H., Terashima Y. et al. (1989) Prostaglandin A and J: antitumor and antiviral prostaglandins. *Adv. Prostaglandin Thromboxane Res.* **19**: 415–418
  - 100 Middleditch B. S. (1975) PGA: fact or artifact? *Prostaglandins* **9**: 409–411
  - 101 Jonsson H. T., Jr, Middleditch B. S., Schexnayder M. A. and Desiderio D. M. (1976) 11,15,19-trihydroxy-9-ketoprost-13-enoic acid and 11,15,19-trihydroxy-9-ketoprost-5,13-dienoic acid in human seminal fluid. *J. Lip. Res.* **17**: 1–6
  - 102 Attalah A., Payakkapan W., Lee J., Carr A. and Brazelton E. (1974) PGA: fact, not artifact. *Prostaglandins* **5**: 69–72
  - 103 Hirata Y., Hayashi H., Ito S., Kikawa Y., Ishibashi M., Sudo M. et al. (1988) Occurrence of 9-deoxy- $\Delta^9,\Delta^{12}$ -13,14-dihydroprostaglandin  $D_2$  in human urine. *J. Biol. Chem.* **263**: 16619–16625
  - 104 Atsmon J., Freeman M. L., Meredith M. J., Sweetman B. J. and Roberts L. J. II. (1990) Conjugation of 9-deoxy- $\Delta^9,\Delta^{12}$ (E)-prostaglandin  $D_2$  with intracellular glutathione and enhancement of its antiproliferative activity by glutathione depletion. *Cancer Res.* **50**: 1879–1885
  - 105 Brame C. J., Salomon R. G., Morrow J. D. and Roberts L. J. II. (1999) Identification of extremely reactive  $\gamma$ -ketoaldehydes (isolevuglandins) as products of the isoprostane pathway and characterization of their lysyl protein adducts. *J. Biol. Chem.* **274**: 13139–13146
  - 106 Salomon R. G., Miller D. B., Zagorski M. G. and Coughlin D. J. (1984) Solvent induced fragmentation of prostaglandin endoperoxides. New aldehyde products from PGH $_2$  and a novel intramolecular 1,2-hydride shift during endoperoxide fragmentation in aqueous solution. *J. Am. Chem. Soc.* **106**: 6049–6060

- 107 Esterbauer H., Schaur R. J. and Zollner H. (1991) Chemistry and biochemistry of 4-hydroxynonenal, malondialdehyde and related aldehydes. *Free Rad. Biol. Med.* **11**: 81–128
- 108 Iyer R. S., Miller D. B. and Salomon R. G. (1990) Decomposition of levuglandin E<sub>2</sub>. Dehydration and allylic rearrangement products. *J. Org. Chem.* **55**: 3175–3180
- 109 Iyer R. S., Kobierski M. E. and Salomon R. (1994) Generation of pyrroles in the reaction of levuglandin E<sub>2</sub> with proteins. *J. Org. Chem.* **59**: 6038–6043
- 110 Smith E. B. and Jensen H. B. (1967) Autoxidation of three 1-alkylpyrroles. *J. Org. Chem.* **32**: 3330–3333.
- 111 Boutand O., Brame C. J., Salomon R. G., Roberts L. J. II and Oates, J. A. (1999) Characterization of lysyl adducts formed from prostaglandin H<sub>2</sub> via the levuglandin pathway. *Biochemistry* **38**: 9389–9396
- 112 Nourooz-Zadeh J., Halliwell B. and Anggard E. E. (1997) Evidence for the formation of F<sub>2</sub>-isoprostanes during peroxidation of eicosapentaenoic acid. *Biochem. Biophys. Res. Comm.* **236**: 467–472
- 113 Skinner E. R., Watt C., Besson J. A. O. and Best P. V. (1993) Differences in the fatty acid composition of the grey and white matter of different regions of the brains of patients with Alzheimer's disease and control subjects. *Brain* **116**: 717–725
- 114 Salem N., Jr, Kim H. Y., and Yergery J. A. (1986) Docosahexaenoic acid: membrane function and metabolism. In: *Health Effects of Polyunsaturated Fatty Acids in Seafoods*, pp 263–317, Simopoulos A. P., Kifer R. R. and Martin R. E. (eds) Academic Press, Orlando, FL.
- 115 Roberts L. J. II, Montine T. J., Markesbery W. R., Tapper A. R., Hardy P., Chemtob S. et al. (1998) Formation of isoprostane-like compounds (Neuroprostanes) in vivo from oxidation of docosahexaenoic acid. *J. Biol. Chem.* **273**: 13605–13612
- 116 Reich E. E., Zackert W. E., Brame C. J., Chen Y., Roberts L. J. II, Hachey D. L. et al. (2000) Formation of novel D-ring and E-ring isoprostane-like compounds (D<sub>4</sub>/E<sub>4</sub>-neuroprostanes) in vivo from docosahexaenoic acid. *Biochemistry* **39**: 2376–2383
- 117 Reich E. E., Markesbery W. R., Roberts L. J. II, Swift L. L., Morrow J. D. and Montine T. J. (2001) Brain regional quantification of F-ring and D-/E-ring isoprostanes and neuroprostanes in Alzheimer's disease. *Am. J. Pathol.* **158**: 293–297
- 118 Nourooz-Zadeh J., Liu E. H., Yhlen b., Anggard E. E. and Halliwell B. (1999) F<sub>4</sub>-isoprostanes as a specific marker of docosahexaenoic acid peroxidation in Alzheimer's disease. *J. Neurochem.* **72**: 734–740
- 119 Bernoud-Hubac N., Davies S. S., Boutaud O., Montine T. J. and Roberts L. J. II (2001) Formation of highly reactive  $\gamma$ -ketoaldehydes (neuroketals) as products of the neuroprostaglandin pathway. *J. Biol. Chem.* **276**: 30964–30970



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